

Connecting via Winsock to STN

Welcome to STN International! Enter x:x

LOGINID:sssptal649axm

PASSWORD:

TERMINAL (ENTER 1, 2, 3, OR ?):2

\* \* \* \* \* Welcome to STN International \* \* \* \* \*

NEWS 1 Web Page URLs for STN Seminar Schedule - N. America  
NEWS 2 "Ask CAS" for self-help around the clock  
NEWS 3 May 10 PROUSDDR now available on STN  
NEWS 4 May 19 PROUSDDR: One FREE connect hour, per account, in both May  
and June 2004  
NEWS 5 May 12 EXTEND option available in structure searching  
NEWS 6 May 12 Polymer links for the POLYLINK command completed in REGISTRY  
NEWS 7 May 17 FRFULL now available on STN  
NEWS 8 May 27 New UPM (Update Code Maximum) field for more efficient patent  
SDIs in CPlus  
NEWS 9 May 27 CPlus super roles and document types searchable in REGISTRY  
NEWS 10 May 27 Explore APOLLIT with free connect time in June 2004  
NEWS 11 Jun 22 STN Patent Forums to be held July 19-22, 2004  
  
NEWS EXPRESS MARCH 31 CURRENT WINDOWS VERSION IS V7.00A, CURRENT  
MACINTOSH VERSION IS V6.0c(ENG) AND V6.0Jc(JP),  
AND CURRENT DISCOVER FILE IS DATED 26 APRIL 2004  
NEWS HOURS STN Operating Hours Plus Help Desk Availability  
NEWS INTER General Internet Information  
NEWS LOGIN Welcome Banner and News Items  
NEWS PHONE Direct Dial and Telecommunication Network Access to STN  
NEWS WWW CAS World Wide Web Site (general information)

Enter NEWS followed by the item number or name to see news on that  
specific topic.

All use of STN is subject to the provisions of the STN Customer  
agreement. Please note that this agreement limits use to scientific  
research. Use for software development or design or implementation  
of commercial gateways or other similar uses is prohibited and may  
result in loss of user privileges and other penalties.

\* \* \* \* \* STN Columbus \* \* \* \* \*

FILE 'HOME' ENTERED AT 09:28:46 ON 23 JUN 2004

=> file agricola caplus biosis

COST IN U.S. DOLLARS	SINCE FILE ENTRY	TOTAL SESSION
FULL ESTIMATED COST	0.63	0.63

FILE 'AGRICOLA' ENTERED AT 09:30:24 ON 23 JUN 2004

FILE 'CAPLUS' ENTERED AT 09:30:24 ON 23 JUN 2004

USE IS SUBJECT TO THE TERMS OF YOUR STN CUSTOMER AGREEMENT.

PLEASE SEE "HELP USAGETERMS" FOR DETAILS.

COPYRIGHT (C) 2004 AMERICAN CHEMICAL SOCIETY (ACS)

FILE 'BIOSIS' ENTERED AT 09:30:24 ON 23 JUN 2004  
COPYRIGHT (C) 2004 BIOLOGICAL ABSTRACTS INC.(R)

=> s phytase

L1 4493 PHYTASE

=> s l1 and yersinia pestis

L2 1 L1 AND YERSINIA PESTIS

=> d ti

L2 ANSWER 1 OF 1 CAPLUS COPYRIGHT 2004 ACS on STN

TI New phytases identified by sequence homology and their use in food processing to lower phytic acid content

=> d so

L2 ANSWER 1 OF 1 CAPLUS COPYRIGHT 2004 ACS on STN

S0 PCT Int. Appl., 208 pp.

CODEN: PIXXD2

=> d pi

L2 ANSWER 1 OF 1 CAPLUS COPYRIGHT 2004 ACS on STN

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	WO 2002048332	A2	20020620	WO 2001-US48774	20011212
	WO 2002048332	C2	20030424		
	WO 2002048332	A3	20030626		
	W:	AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM			
	RW:	GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG			
	AU 2002030953	A5	20020624	AU 2002-30953	20011212
	US 2003101476	A1	20030529	US 2001-21723	20011212

=> s l1 and (cdna or gene or coding region)

L3 482 L1 AND (CDNA OR GENE OR CODING REGION)

=> s l3 and food composition

L4 0 L3 AND FOOD COMPOSITION

=> s l3 and food

L5 73 L3 AND FOOD

=> s l3 and synthetic

L6 35 L3 AND SYNTHETIC

=> dup rem l6

PROCESSING COMPLETED FOR L6

L7 28 DUP REM L6 (7 DUPLICATES REMOVED)

=> d 1-10 ti

L7 ANSWER 1 OF 28 CAPLUS COPYRIGHT 2004 ACS on STN

TI Isolation, characterization, and molecular cloning of the cDNA

encoding a novel **phytase** from *Aspergillus niger* 113 and high expression in *Pichia pastoris*

- L7 ANSWER 2 OF 28 CAPLUS COPYRIGHT 2004 ACS on STN  
TI Thermotolerant **phytase** expression in plants and isolation for use in animal feed and food processing
- L7 ANSWER 3 OF 28 CAPLUS COPYRIGHT 2004 ACS on STN  
TI Constitutive promoters of *Arabidopsis* and their use in expression of foreign genes in transgenic plants
- L7 ANSWER 4 OF 28 CAPLUS COPYRIGHT 2004 ACS on STN  
TI Production, expression and sequence of modified recombinant *Escherichia coli* **phytase** with enhanced thermostability and proteolytic resistance for use in foodstuffs
- L7 ANSWER 5 OF 28 CAPLUS COPYRIGHT 2004 ACS on STN  
TI Continuous fermentation system and production of engineered phytases with improved thermostability
- L7 ANSWER 6 OF 28 CAPLUS COPYRIGHT 2004 ACS on STN  
TI Constitutive promoters of *Arabidopsis* and their use in expression of foreign genes in transgenic plants
- L7 ANSWER 7 OF 28 CAPLUS COPYRIGHT 2004 ACS on STN DUPLICATE 1  
TI Fungal *phyA* **gene** expressed in potato leaves produces active and stable **phytase**
- L7 ANSWER 8 OF 28 CAPLUS COPYRIGHT 2004 ACS on STN  
TI Engineering the root-soil interface via targeted expression of a **synthetic phytase gene** in trichoblasts
- L7 ANSWER 9 OF 28 CAPLUS COPYRIGHT 2004 ACS on STN  
TI A flocculation-centrifugation method of purifying  $\alpha$ -amylase, cellulase and other enzymes, and design of  $\alpha$ -amylase with increased activity and stability
- L7 ANSWER 10 OF 28 CAPLUS COPYRIGHT 2004 ACS on STN  
TI Sequences of myo-inositol oxygenases for the production of glucuronic acids, ascorbic acids, and glucaric acids

=> d 1-2 ab

- L7 ANSWER 1 OF 28 CAPLUS COPYRIGHT 2004 ACS on STN  
AB Phytases catalyze the release of phosphate from phytic acid. **Phytase**-producing microorganisms were selected by culturing the soil exts. on agar plates containing phytic acid. Two hundred colonies that exhibited potential **phytase** activity were selected for further study. The colony showing the highest **phytase** activity was identified as *Aspergillus niger* and designated strain 113. The **phytase gene** from *A. niger* 113 (*phyI1*) was isolated, cloned, and characterized. The nucleotide and deduced amino acid sequence identity between *phyI1* and *phyA* from NRRL3135 were 90% and 98%, resp. The identity between *phyI1* and *phyA* from SK-57 was 89% and 96%. A **synthetic phytase gene**, *phyI1s*, was synthesized by successive PCR and transformed into the yeast expression vector carrying a signal peptide that was designed and synthesized using *P. pastoris* biased codon. For the **phytase** expression and secretion, the construct was integrated into the genome of *P. pastoris* by homologous recombination. Over-expressing strains were selected and fermented. It was discovered that .apprx.4.2 g **phytase** could be purified from one liter of culture fluid. The activity of the resulting **phytase** was 9.5 U/mg. Due to the heavy glycosylation, the

expressed **phytase** varied in size (120, 95, 85, and 64 kDa), but could be deglycosylated to a homogeneous 64 kDa species. An enzymic kinetics anal. showed that the **phytase** had two pH optima (pH 2.0 and pH 5.0) and an optimum temperature of 60°C.

L7 ANSWER 2 OF 28 CAPLUS COPYRIGHT 2004 ACS on STN

AB The invention provides a **synthetic phytase** polynucleotide which is optimized for expression in plants and which encodes a thermotolerant **phytase**, as well as isolated thermotolerant **phytase** enzyme. Also provided are feed or food products comprising a thermotolerant **phytase**, and transgenic plants which express the thermotolerant **phytase**. Further provided are methods for making and using thermotolerant phytases, e.g., a method of using a thermotolerant **phytase** in feed and food processing.

=> d so

L7 ANSWER 1 OF 28 CAPLUS COPYRIGHT 2004 ACS on STN

SO Journal of Biochemistry and Molecular Biology (2004), 37(3), 282-291  
CODEN: JBMBE5; ISSN: 1225-8687

=> d 2 pi

L7 ANSWER 2 OF 28 CAPLUS COPYRIGHT 2004 ACS on STN

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2003057248	A1	20030717	WO 2002-US41787	20021230
W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, SI, SK, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG				
US 2003170293	A1	20030911	US 2002-334671	20021230

=> d 4 ab

L7 ANSWER 4 OF 28 CAPLUS COPYRIGHT 2004 ACS on STN

AB The invention provides isolated and recombinant **phytase** enzymes. In one aspect, the **phytase** is produced by modification of the wild type **gene** appA of Escherichia coli by using **gene** site-saturation mutagenesis. The nucleotide sequence and the encoded amino acid sequence of the modified E. coli **phytase** are disclosed. The enzyme has **phytase** activity and improved thermal tolerance as compared with the wild-type enzyme. In addition, the enzyme has improved protease stability at low pH. Glycosylation of the modified **phytase** provided a further improved enzyme having improved thermal tolerance and protease stability. The enzyme of the invention can be produced from recombinant host cells, namely E. coli, Pichia pastoris and Schizosaccharomyces pombe. The phytases of the invention can be used to aid in the digestion of phytate where desired. In particular, the phytases of the invention can be used in foodstuffs to improve the feeding value of phytate rich ingredients. Also provided are methods for obtaining a variant polynucleotide encoding a **phytase** and for obtaining a **phytase** with enhanced thermostability or

thermotolerance at high or low temps.

=> d 4 so

L7 ANSWER 4 OF 28 CAPLUS COPYRIGHT 2004 ACS on STN  
SO U.S. Pat. Appl. Publ., 113 pp., Cont.-in-part of U.S. Ser. No. 866,379.  
CODEN: USXXCO

=> d 4 pi

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	US 2003103958	A1	20030605	US 2002-156660	20020524
	US 5876997	A	19990302	US 1997-910798	19970813
	US 6110719	A	20000829	US 1999-259214	19990301
	US 6190897	B1	20010220	US 1999-291931	19990413
	US 6183740	B1	20010206	US 1999-318528	19990525
	US 6720014	B1	20040413	US 2000-580515	20000525
	US 2002136754	A1	20020926	US 2001-866379	20010524

=> d 4 in

L7 ANSWER 4 OF 28 CAPLUS COPYRIGHT 2004 ACS on STN  
IN Short, Jay M.; Kretz, Keith; Gray, Kevin A.; Barton, Nelson R.; Garrett,  
James B.; O'Donoghue, Eileen; Mathur, Eric J.

=> d 5 ab

L7 ANSWER 5 OF 28 CAPLUS COPYRIGHT 2004 ACS on STN  
AB A fermentation assembly is provided comprising: (a) a vessel for culturing living cells; (b) at least two storage flasks in fluid communication with the vessel for supply of liqs. and a first transport means for transferring the liqs. from the storage flasks to the vessel; (c) individual appliances operably connected to the transport means for monitoring the supply of the contents of the storage flasks to the vessel; (d) a harvest flask in fluid communication with the vessel and a second transport means for transferring the fermentation broth from the vessel to the harvest flask; and (e) a device operably connected to the first transport means for controlling and maintaining a constant dilution rate in the vessel with varying rates of individual supply of liquid from the storage flasks to the vessel. Splitting of cultivation media used in a continuous fermentation process allows one to study the influence on growth and metabolite-production of microorganisms, and thus to determine optimal conditions for the fermentation

process. Such a fermentation assembly device and process for recombinant **phytase** production by *Hansenula polymorpha* resulted in an optimized productivity of 0.094 g **phytase** per L per h; biomass in outflow was 56.8 g/L. The invention also relates to phytases with improved thermostability, where stabilizing amino acid mutations are introduced into a homologous protein, or the active site of a **phytase** is replaced in part or in total. Specific variants of *Aspergillus fumigatus* **phytase** and of consensus phytases are disclosed.

=> d 5 so

L7 ANSWER 5 OF 28 CAPLUS COPYRIGHT 2004 ACS on STN  
SO U.S., 204 pp.  
CODEN: USXXAM

=> d 5 pi

L7 ANSWER 5 OF 28 CAPLUS COPYRIGHT 2004 ACS on STN  
PATENT NO. KIND DATE APPLICATION NO. DATE  
-----  
PI US 6599735 B1 20030729 US 2000-684855 20001006  
EP 1092764 A3 20040317 EP 2000-121663 20001004  
EP 1092764 A2 20010418  
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT,  
IE, SI, LT, LV, FI, RO, MK, CY, AL  
AU 2000062501 A5 20010412 AU 2000-62501 20001005  
CA 2319658 AA 20010411 CA 2000-2319658 20001006  
BR 2000005170 A 20010529 BR 2000-5170 20001010  
JP 2001145480 A2 20010529 JP 2000-311300 20001011  
CN 1303921 A 20010718 CN 2000-135543 20001011  
US 2003224491 A1 20031204 US 2003-442538 20030520

=> d 7-8 ab

L7 ANSWER 7 OF 28 CAPLUS COPYRIGHT 2004 ACS on STN DUPLICATE 1  
AB Fungal **phyA gene** from *Aspergillus ficuum* (niger) was cloned and expressed in potato leaves. The recombinant enzyme was stable and catalytically active. The expressed protein in the leaves of the dicotyledonous plant retained most phys. and catalytic properties of the benchmark *A. ficuum* **phytase**. The expressed enzyme was, however, 15% less glycosylated than the native **phytase**. The usual bi-hump pH optima profile, which is characteristic of the fungal **phytase**, was altered; however, the pH optimum at 5.0 was unchanged for phytate and at 4.0 for **synthetic** substrate p-nitrophenyl phosphate. The temperature was, however, unchanged. The expressed **phytase** was found to be as sensitive as the native enzyme to the inhibitory action of pseudo substrate, myo-inositol hexasulfate, while losing about 90% of the activity at 20  $\mu$ M inhibitor concentration. Similar to the benchmark **phytase**, the expressed **phytase** in leaves was completely inactivated by Arg modifier phenylglyoxal at 60 nM. In addition, the expressed **phytase** in the leaves was inhibited by antibody raised against a 20-mer internal peptide, which is present on the surface of the mol. as shown by the x-ray deduced 3D structure of fungal **phytase**. Taken together, the biochem. evidences indicate that fungal **phytase** when cloned and expressed in potato leaves produces a stable and active biocatalyst. Biofarming, therefore, is an alternative way to produce functional hydrolytic enzymes as exemplified by the expression of *A. ficuum* (niger) **phyA gene** in potato leaf.

L7 ANSWER 8 OF 28 CAPLUS COPYRIGHT 2004 ACS on STN  
AB For biochem. modification of the root-soil interface, the engineered secretion of stable enzymes from trichoblasts (= root hair bearing rhizodermal cells) is proposed. As a reporter activity, we chose to express a **synthetic gene** encoding a secretory **phytase** (PHY) directed by a trichoblast-specific promoter in root hair cells of the crop plant potato. Transgenic plants, produced and secreted **phytase** in sufficient amts. to release phosphate from phytate in liquid medium. When grown in an unsterile substrate containing phytate, transgenic plants accumulated 40% more P in leaves than wild-type plants. The improved P nutrition driven by trichoblast-targeted expression and subsequent secretion of PHY illustrates the potential of using trichoblast-targeted expression of suitable enzymes for future applications in plant nutrition, phytoremediation and mol. farming.

=> d 7-8 ti

L7 ANSWER 7 OF 28 CAPLUS COPYRIGHT 2004 ACS on STN DUPLICATE 1  
TI Fungal phyA **gene** expressed in potato leaves produces active and stable **phytase**

L7 ANSWER 8 OF 28 CAPLUS COPYRIGHT 2004 ACS on STN  
TI Engineering the root-soil interface via targeted expression of a **synthetic phytase gene** in trichoblasts

=> d 7-8 so

L7 ANSWER 7 OF 28 CAPLUS COPYRIGHT 2004 ACS on STN DUPLICATE 1  
S0 Biochemical and Biophysical Research Communications (2003), 306(2), 603-609  
CODEN: BBRCA9; ISSN: 0006-291X

L7 ANSWER 8 OF 28 CAPLUS COPYRIGHT 2004 ACS on STN  
S0 Plant Biotechnology Journal (2003), 1(5), 353-360  
CODEN: PBJLAE; ISSN: 1467-7644

=> d 11-20 ti

L7 ANSWER 11 OF 28 AGRICOLA Compiled and distributed by the National Agricultural Library of the Department of Agriculture of the United States of America. It contains copyrighted materials. All rights reserved. (2004) on STN DUPLICATE 2  
TI In vitro properties of phytases from various microbial origins.

L7 ANSWER 12 OF 28 CAPLUS COPYRIGHT 2004 ACS on STN DUPLICATE 3  
TI High expression of a heat-stable **phytase** in *Pichia pastoris*

L7 ANSWER 13 OF 28 CAPLUS COPYRIGHT 2004 ACS on STN DUPLICATE 4  
TI The consensus concept for thermostability engineering of proteins: further proof of concept

L7 ANSWER 14 OF 28 CAPLUS COPYRIGHT 2004 ACS on STN  
TI Codon usage in methylotrophic yeasts and its use in increasing yields of foreign proteins in yeast expression hosts

L7 ANSWER 15 OF 28 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN  
TI Cloning and sequence analysis of the **phytase phyA gene** of *Aspergillus niger* N25.

L7 ANSWER 16 OF 28 CAPLUS COPYRIGHT 2004 ACS on STN  
TI Overexpression of artificial **synthetic gene** of *Aspergillus niger* NRRL3135 **Phytase** in *Pichia pastoris*

L7 ANSWER 17 OF 28 CAPLUS COPYRIGHT 2004 ACS on STN  
TI Transgenic animals expressing salivary proteins

L7 ANSWER 18 OF 28 CAPLUS COPYRIGHT 2004 ACS on STN  
TI Fungal phytases engineered for improved thermostability and related properties

L7 ANSWER 19 OF 28 CAPLUS COPYRIGHT 2004 ACS on STN  
TI **Phytase** consensus forms and muteins and formulations for improved thermostability

L7 ANSWER 20 OF 28 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN  
TI Exchanging the active site between phytases for altering the functional properties of the enzyme.

=> d 17 ab

L7 ANSWER 17 OF 28 CAPLUS COPYRIGHT 2004 ACS on STN

AB The invention provides a transgenic animal having within its genome a transgene construct for salivary gland-specific or gastrointestinal tract-specific expression of a protein. In a preferred embodiment, the protein is a **phytase** or a homolog thereof (e.g., bifunctional **phytase**/acid phosphatase encoded by the appA gene of *Escherichia coli*) in order to enable animals to utilize available phytate in feed, thereby improving both phosphorus nutrition and decreasing environmental pollution. Such proteins may be heterologous and may be specifically expressed in the salivary gland of the animal by operably linking the nucleic acid sequence encoding the protein with regulatory sequence including a salivary gland protein promoter/enhancer. Salivary gland-specific expression also provides a means for achieving glycosylation of the recombinant protein. Promoters/enhancers specific for salivary expression include those regulating the genes encoding parotid secretory protein (PSP) of mouse, proline-rich protein (PRP) of rat, or salivary amylase. Also provided are methods of expressing and producing proteins using such nucleic acid constructs. Further, antibodies specific to such proteins and immunol. diagnostic kits are also provided.

=> d 17 pi

L7 ANSWER 17 OF 28 CAPLUS COPYRIGHT 2004 ACS on STN

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2000064247	A1	20001102	WO 2000-CA430	20000420
W: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG				
EP 1170994	A1	20020116	EP 2000-920304	20000420
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO				

=> d 19 ab

L7 ANSWER 19 OF 28 CAPLUS COPYRIGHT 2004 ACS on STN

AB A major barrier to the wide use of phytases is the constraint of thermal stability (80-120°) required for these enzymes to withstand inactivation during feed processes; the currently available industrial phytases all originate from *Aspergillus niger* and have low intrinsic resistance to heat inactivation. Consensus **phytase** sequences are prepared by alignment of the amino acid sequences of multiple *Aspergillus*, *Basidiomycetes*, and related fungal phytases, determination of the consensus sequence, and synthesis of a **synthetic gene** for expression of the desired consensus sequence in recombinant *Aspergillus niger*, *Saccharomyces cerevisiae*, and/or *Hansenula polymorpha*. Specific amino acid residues that appear associated with improved thermostability are also constructed by site-directed mutagenesis. Stabilized **phytase** formulations are disclosed with comprise **phytase** and at least one stabilizing agent selected from the group consisting of: (a) C5 sugars such as xylitol and ribitol; (b) polyethylene glycol having a mol. weight of 600-4000 Da; (c) the disodium salts of



malonic, succinic, and glutaric acid; (d) CM-cellulose; and (e) sodium alginate. Alternatively, **phytase** may be stabilized by chemical crosslinking with either glutaraldehyde, or oxidn of **phytase** carbohydrate residues with sodium periodate and subsequent addition of adipic acid dihydrazide. Another aspect of the present invention concerns methods of preparing feed compns. for monogastric animals, whereby the feed is supplemented with a thermostabilized dry or liquid enzyme formulation.

=> d 10 so

L7 ANSWER 10 OF 28 CAPLUS COPYRIGHT 2004 ACS on STN  
S0 PCT Int. Appl., 127 pp.  
CODEN: PIXXD2

=> d 19 so

L7 ANSWER 19 OF 28 CAPLUS COPYRIGHT 2004 ACS on STN  
S0 Eur. Pat. Appl., 103 pp.  
CODEN: EPXXDW

=> d 19 pi

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
EP 969089	A1	20000105	EP 1999-111949	19990623
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO				
AU 9936750	A1	20000210	AU 1999-36750	19990624
AU 760737	B2	20030522		
CN 1241634	A	20000119	CN 1999-108867	19990628
KR 2000006528	A	20000125	KR 1999-24946	19990628
BR 9903286	A	20000516	BR 1999-3286	19990628
JP 2000053584	A2	20000222	JP 1999-184484	19990629

=> d 20 ab

L7 ANSWER 20 OF 28 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN  
AB By using a novel consensus approach, we have previously managed to generate a fully **synthetic phytase**, consensus **phytase-1**, that was 15-26 degreeC more thermostable than the parent fungal phytases used in its design (Lehmann et al., 2000). We now sought to use the backbone of consensus **phytase-1** and to modify its catalytic properties. This was done by replacing a considerable part of the active site (i.e., all the divergent residues) with the corresponding residues of *Aspergillus niger* NRRL 3135 **phytase**, which displays pronounced differences in specific activity, substrate specificity, and pH-activity profile. For the new protein termed consensus **phytase-7**, a major-although not complete-shift in catalytic properties was observed, demonstrating that rational transfer of favorable catalytic properties from one **phytase** to another is possible by using this approach. Although the exchange of the active site was associated with a 7.6 degreeC decrease in unfolding temperature (Tm) as measured by differential scanning calorimetry, consensus **phytase-7** still was >7 degreeC more thermostable than all wild-type ascomycete phytases known to date. Thus, combination of the consensus approach with the selection of a "preferred" active site allows the design of a thermostabilized variant of an enzyme family of interest that (most closely) matches the most favorable catalytic properties found among its family members.

=> d 20 so

L7 ANSWER 20 OF 28 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN  
SO Protein Science, (October, 2000) Vol. 9, No. 10, pp. 1866-1872. print.  
ISSN: 0961-8368.

=> d 21-28 ti

L7 ANSWER 21 OF 28 AGRICOLA Compiled and distributed by the National  
Agricultural Library of the Department of Agriculture of the United States  
of America. It contains copyrighted materials. All rights reserved.  
(2004) on STN DUPLICATE 5

TI The consensus concept for thermostability engineering of proteins.

L7 ANSWER 22 OF 28 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN  
TI Expression of *Bacillus subtilis* **phytase** in *Lactobacillus*  
*plantarum* 755.

L7 ANSWER 23 OF 28 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN  
TI Characterization and overproduction of the *Escherichia coli* appA encoded  
bifunctional enzyme that exhibits both **phytase** and acid  
phosphatase activities.

L7 ANSWER 24 OF 28 CAPLUS COPYRIGHT 2004 ACS on STN  
TI Cryptic regulatory elements obtained from plants for seed specific  
expression in both dicots and monocots

L7 ANSWER 25 OF 28 CAPLUS COPYRIGHT 2004 ACS on STN  
TI Thermostable phytases, genes encoding them and transgenic plants  
expressing the genes for use in low-phytate feed preparation

L7 ANSWER 26 OF 28 CAPLUS COPYRIGHT 2004 ACS on STN  
TI An in vivo recombination method for increased efficiency of preparation of  
active variants of proteins

L7 ANSWER 27 OF 28 CAPLUS COPYRIGHT 2004 ACS on STN  
TI Generation of variant polypeptides by in vivo recombination between linear  
DNAs

L7 ANSWER 28 OF 28 CAPLUS COPYRIGHT 2004 ACS on STN  
TI Transformation and selection of maize tissue and the regeneration of  
stably transformed fertile plants

=> d 21 ab

L7 ANSWER 21 OF 28 AGRICOLA Compiled and distributed by the National  
Agricultural Library of the Department of Agriculture of the United States  
of America. It contains copyrighted materials. All rights reserved.  
(2004) on STN DUPLICATE 5

AB Previously, sequence comparisons between a mesophilic enzyme and a more  
thermostable homologue were shown to be a feasible approach to  
successfully predict thermostabilizing amino acid substitutions. The  
'consensus approach' described in the present paper shows that even a set  
of amino acid sequences of homologous, mesophilic enzymes contains  
sufficient information to allow rapid design of a thermostabilized, fully  
functional variant of this family of enzymes. A sequence alignment of  
homologous fungal phytases was used to calculate a consensus  
**phytase** amino acid sequence. Upon construction of the  
**synthetic gene**, recombinant expression and purification,  
the first **phytase** obtained, termed consensus **phytase**

-1, displayed an unfolding temperature (T<sub>m</sub>) of 78.0 degrees C which is 15-22 degrees C higher than the T<sub>m</sub> values of all parent phytases used in its design. Refinement of the approach, combined with site-directed mutagenesis experiments, yielded optimized consensus phytases with T<sub>m</sub> values of up to 90.4 degrees C. These increases in T<sub>m</sub> are due to the combination of multiple amino acid exchanges which are distributed over the entire sequence of the protein and mainly affect surface-exposed residues; each individual substitution has a rather small thermostabilizing effect only. Remarkably, in spite of the pronounced increase in thermostability, catalytic activity at 37 degrees C is not compromised. Thus, the design of consensus proteins is a potentially powerful and novel alternative to directed evolution and to a series of rational approaches for thermostability engineering of enzymes and other proteins.

=> d 21 so

- L7 ANSWER 21 OF 28 AGRICOLA Compiled and distributed by the National Agricultural Library of the Department of Agriculture of the United States of America. It contains copyrighted materials. All rights reserved.  
(2004) on STN DUPLICATE 5
- SO Biochimica et biophysica acta = International journal of biochemistry and biophysics, Dec 29, 2000. Vol. 1543, No. 2. p. 408-415  
Publisher: Amsterdam : Elsevier Science B.V.  
CODEN: BBACAQ; ISSN: 0006-3002

=> d 25 ab

- L7 ANSWER 25 OF 28 CAPLUS COPYRIGHT 2004 ACS on STN
- AB The use of thermostable phytases in the preparation of animal feed low in phytic acid is described. A thermostable **phytase** can be included feed processing before or during agglomeration and will continue to function during thermal processing of the raw material. A transgenic plant expressing the **gene** for a thermostable **phytase** may be used directly in the feed. Consensus sequences for thermostable phytases are developed using a combination of temperature optima, differential scanning calorimetry, and sequence comparison.

=> d 25 pi

- L7 ANSWER 25 OF 28 CAPLUS COPYRIGHT 2004 ACS on STN
- | PATENT NO.  | KIND | DATE     | APPLICATION NO. | DATE     |
|---|------|----------|-----------------|----------|
| WO 9948380  | A1   | 19990930 | WO 1999-DK154   | 19990322 |
| W: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM |      |          |                 |          |
| RW: GH, GM, KE, LS, MW, SD, SL, SZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG  |      |          |                 |          |
| CA 2325440  | AA   | 19990930 | CA 1999-2325440 | 19990322 |
| AU 9933267  | A1   | 19991018 | AU 1999-33267   | 19990322 |
| AU 753475   | B2   | 20021017 |                 |          |
| BR 9909006  | A    | 20001128 | BR 1999-9006    | 19990322 |
| EP 1065941  | A1   | 20010110 | EP 1999-914443  | 19990322 |
| R: BE, CH, DE, DK, ES, FR, GB, IT, LI, NL, SE, IE   |      |          |                 |          |
| JP 2002508942   | T2   | 20020326 | JP 2000-537445  | 19990322 |
| US 6514495  | B1   | 20030204 | US 1999-273871  | 19990322 |

US 2002127218  
US 6689358

A1 20020912  
B2 20040210

US 2002-83452 20020226

=> s ((short j?) or (short, j?))/au  
L8 1091 ((SHORT J?) OR (SHORT, J?))/AU

=> s l8 and phytase  
L9 11 L8 AND PHYTASE

=> dup rem l9  
PROCESSING COMPLETED FOR L9  
L10 9 DUP REM L9 (2 DUPLICATES REMOVED)

=> d 1-9 ti

L10 ANSWER 1 OF 9 CAPLUS COPYRIGHT 2004 ACS on STN DUPLICATE 1  
TI Protein and cDNA sequences of a Escherichia coli B. **Phytase** and use in foodstuffs to improve the feeding value of phytate rich ingredients

L10 ANSWER 2 OF 9 CAPLUS COPYRIGHT 2004 ACS on STN  
TI Sequences of modified recombinant Escherichia coli phytases and methods of making and using them

L10 ANSWER 3 OF 9 CAPLUS COPYRIGHT 2004 ACS on STN  
TI Enhancing the thermal tolerance and gastric performance of a microbial **phytase** for use as a phosphate-mobilizing monogastric-feed supplement

L10 ANSWER 4 OF 9 CAPLUS COPYRIGHT 2004 ACS on STN  
TI Production, expression and sequence of modified recombinant Escherichia coli **phytase** with enhanced thermostability and proteolytic resistance for use in foodstuffs

L10 ANSWER 5 OF 9 CAPLUS COPYRIGHT 2004 ACS on STN  
TI New phytases identified by sequence homology and their use in food processing to lower phytic acid content

L10 ANSWER 6 OF 9 CAPLUS COPYRIGHT 2004 ACS on STN  
TI Recombinant bacterial phytases and their use for degradation of food phytates.

L10 ANSWER 7 OF 9 CAPLUS COPYRIGHT 2004 ACS on STN DUPLICATE 2  
TI Recombinant **phytase** from Escherichia coli B and its use for improving nutritional value of phytate-containing animal feed

L10 ANSWER 8 OF 9 CAPLUS COPYRIGHT 2004 ACS on STN  
TI Recombinant bacterial phytases and uses for improved nutritional value of phytate-containing foodstuffs

L10 ANSWER 9 OF 9 CAPLUS COPYRIGHT 2004 ACS on STN  
TI Recombinant bacterial phytases and their uses for improving nutritional value of phytate-containing feed

=> d 1-9 so

L10 ANSWER 1 OF 9 CAPLUS COPYRIGHT 2004 ACS on STN DUPLICATE 1  
SO U.S., 48 pp., Cont.-in-part of U.S. 6,183,740.  
CODEN: USXXAM

L10 ANSWER 2 OF 9 CAPLUS COPYRIGHT 2004 ACS on STN  
SO U.S. Pat. Appl. Publ., 74 pp., Cont.-in-part of U.S. Ser. No. 866,379.  
CODEN: USXXCO

L10 ANSWER 3 OF 9 CAPLUS COPYRIGHT 2004 ACS on STN  
SO Applied and Environmental Microbiology (2004), 70(5), 3041-3046  
CODEN: AEMIDF; ISSN: 0099-2240

L10 ANSWER 4 OF 9 CAPLUS COPYRIGHT 2004 ACS on STN  
 SO U.S. Pat. Appl. Publ., 113 pp., Cont.-in-part of U.S. Ser. No. 866,379.  
 CODEN: USXXCO

L10 ANSWER 5 OF 9 CAPLUS COPYRIGHT 2004 ACS on STN  
 SO PCT Int. Appl., 208 pp.  
 CODEN: PIXXD2

L10 ANSWER 6 OF 9 CAPLUS COPYRIGHT 2004 ACS on STN  
 SO U.S. Pat. Appl. Publ., 62 pp., Cont.-in-part of U.S. Ser. No. 580,515.  
 CODEN: USXXCO

L10 ANSWER 7 OF 9 CAPLUS COPYRIGHT 2004 ACS on STN DUPLICATE 2  
 SO U.S., 33 pp., Cont.-in-part of U.S. Ser. No. 291,931.  
 CODEN: USXXAM

L10 ANSWER 8 OF 9 CAPLUS COPYRIGHT 2004 ACS on STN  
 SO PCT Int. Appl., 170 pp.  
 CODEN: PIXXD2

L10 ANSWER 9 OF 9 CAPLUS COPYRIGHT 2004 ACS on STN  
 SO PCT Int. Appl., 147 pp.  
 CODEN: PIXXD2

=> d 1-9 pi

L10 ANSWER 1 OF 9 CAPLUS COPYRIGHT 2004 ACS on STN DUPLICATE 1

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
US 6720014	B1	20040413	US 2000-580515	20000525
US 5876997	A	19990302	US 1997-910798	19970813
US 6110719	A	20000829	US 1999-259214	19990301
US 6190897	B1	20010220	US 1999-291931	19990413
US 6183740	B1	20010206	US 1999-318528	19990525
WO 2001090333	A2	20011129	WO 2001-US17118	20010524
WO 2001090333	A3	20030123		
W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG				
US 2002136754	A1	20020926	US 2001-866379	20010524
EP 1301592	A2	20030416	EP 2001-937752	20010524
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO, MK, CY, AL, TR				
JP 2003534009	T2	20031118	JP 2001-587129	20010524
BR 2001011124	A	20040225	BR 2001-11124	20010524
US 2003049815	A1	20030313	US 2001-34985	20011221
US 2003103958	A1	20030605	US 2002-156660	20020524
US 2003232041	A1	20031218	US 2003-430356	20030505
US 2004091968	A1	20040513	US 2003-601319	20030620

L10 ANSWER 2 OF 9 CAPLUS COPYRIGHT 2004 ACS on STN

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
US 2004091968	A1	20040513	US 2003-601319	20030620
US 5876997	A	19990302	US 1997-910798	19970813
US 6110719	A	20000829	US 1999-259214	19990301

US 6190897	B1	20010220	US 1999-291931	19990413
US 6183740	B1	20010206	US 1999-318528	19990525
US 6720014	B1	20040413	US 2000-580515	20000525
US 2002136754	A1	20020926	US 2001-866379	20010524

L10 ANSWER 3 OF 9 CAPLUS COPYRIGHT 2004 ACS on STN

L10	ANSWER 4 OF 9	CAPLUS	COPYRIGHT 2004	ACS on STN	
	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
	-----	----	-----	-----	-----
PI	US 2003103958	A1	20030605	US 2002-156660	20020524
	US 5876997	A	19990302	US 1997-910798	19970813
	US 6110719	A	20000829	US 1999-259214	19990301
	US 6190897	B1	20010220	US 1999-291931	19990413
	US 6183740	B1	20010206	US 1999-318528	19990525
	US 6720014	B1	20040413	US 2000-580515	20000525
	US 2002136754	A1	20020926	US 2001-866379	20010524

L10	ANSWER 5 OF 9	CAPLUS	COPYRIGHT 2004	ACS on STN	
	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
	-----	----	-----	-----	-----
PI	WO 2002048332	A2	20020620	WO 2001-US48774	20011212
	WO 2002048332	C2	20030424		
	WO 2002048332	A3	20030626		

W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM

RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG

AU 2002030953	A5	20020624	AU 2002-30953	20011212
US 2003101476	A1	20030529	US 2001-21723	20011212

L10	ANSWER 6 OF 9	CAPLUS	COPYRIGHT 2004	ACS on STN	
	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
	-----	----	-----	-----	-----
PI	US 2002136754	A1	20020926	US 2001-866379	20010524
	US 5876997	A	19990302	US 1997-910798	19970813
	US 6110719	A	20000829	US 1999-259214	19990301
	US 6190897	B1	20010220	US 1999-291931	19990413
	US 6183740	B1	20010206	US 1999-318528	19990525
	US 6720014	B1	20040413	US 2000-580515	20000525
	WO 2002095003	A2	20021128	WO 2002-US16482	20020524

W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM

RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG

US 2003103958	A1	20030605	US 2002-156660	20020524
US 2004091968	A1	20040513	US 2003-601319	20030620

L10	ANSWER 7 OF 9	CAPLUS	COPYRIGHT 2004	ACS on STN	DUPLICATE 2
	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
	-----	----	-----	-----	-----
PI	US 6183740	B1	20010206	US 1999-318528	19990525
	US 5876997	A	19990302	US 1997-910798	19970813

US 6110719	A	20000829	US 1999-259214	19990301
US 6190897	B1	20010220	US 1999-291931	19990413
WO 2000071728	A1	20001130	WO 2000-US14846	20000525
W: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG				
EP 1180152	A1	20020220	EP 2000-937932	20000525
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO				
BR 2000010946	A	20020416	BR 2000-10946	20000525
JP 2003500057	T2	20030107	JP 2000-620105	20000525
US 6720014	B1	20040413	US 2000-580515	20000525
US 2001055788	A1	20011227	US 2001-777566	20010205
US 2002136754	A1	20020926	US 2001-866379	20010524
ZA 2001009428	A	20030317	ZA 2001-9428	20011115
US 2003049815	A1	20030313	US 2001-34985	20011221
US 2003103958	A1	20030605	US 2002-156660	20020524
US 2003232041	A1	20031218	US 2003-430356	20030505
US 2004091968	A1	20040513	US 2003-601319	20030620

L10 ANSWER 8 OF 9 CAPLUS COPYRIGHT 2004 ACS on STN

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
-----				
PI WO 2001090333	A2	20011129	WO 2001-US17118	20010524
WO 2001090333	A3	20030123		
W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG				
US 6720014	B1	20040413	US 2000-580515	20000525
EP 1301592	A2	20030416	EP 2001-937752	20010524
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO, MK, CY, AL, TR				
JP 2003534009	T2	20031118	JP 2001-587129	20010524
BR 2001011124	A	20040225	BR 2001-11124	20010524

L10 ANSWER 9 OF 9 CAPLUS COPYRIGHT 2004 ACS on STN

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
-----				
PI WO 2000071728	A1	20001130	WO 2000-US14846	20000525
W: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG				
US 6183740	B1	20010206	US 1999-318528	19990525
EP 1180152	A1	20020220	EP 2000-937932	20000525
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO				



BR 2000010946	A	20020416	BR 2000-10946	20000525
JP 2003500057	T2	20030107	JP 2000-620105	20000525

```
=> s ((mathur, e?) or (mathur e?))/au
L11      126 ((MATHUR, E?) OR (MATHUR E?))/AU
```

```
=> s l11 and phytase
L12      3 L11 AND PHYTASE
```

```
=> dup rem l12
PROCESSING COMPLETED FOR L12
L13      3 DUP REM L12 (0 DUPLICATES REMOVED)
```

```
=> d 1-3 ti
```

```
L13 ANSWER 1 OF 3 CAPLUS COPYRIGHT 2004 ACS on STN
TI Production, expression and sequence of modified recombinant Escherichia
coli phytase with enhanced thermostability and proteolytic
resistance for use in foodstuffs
```

```
L13 ANSWER 2 OF 3 CAPLUS COPYRIGHT 2004 ACS on STN
TI New phytases identified by sequence homology and their use in food
processing to lower phytic acid content
```

```
L13 ANSWER 3 OF 3 CAPLUS COPYRIGHT 2004 ACS on STN
TI Recombinant bacterial phytases and their use for degradation of food
phytates.
```

```
=> s ((richardson, t?) or (richardson t?))/AU
L14      1315 ((RICHARDSON, T?) OR (RICHARDSON T?))/AU
```

```
=> s l14 and phytase
L15      1 L14 AND PHYTASE
```

```
=> d ti
```

```
L15 ANSWER 1 OF 1 CAPLUS COPYRIGHT 2004 ACS on STN
TI New phytases identified by sequence homology and their use in food
processing to lower phytic acid content
```

```
=> d pi
```

```
L15 ANSWER 1 OF 1 CAPLUS COPYRIGHT 2004 ACS on STN
PATENT NO.      KIND  DATE      APPLICATION NO.  DATE
-----
PI WO 2002048332    A2   20020620    WO 2001-US48774  20011212
  WO 2002048332    C2   20030424
  WO 2002048332    A3   20030626
    W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN,
      CO, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM,
      HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS,
      LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO,
      RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN,
      YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM
    RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AT, BE, CH,
      CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR,
      BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG
AU 2002030953    A5   20020624    AU 2002-30953    20011212
US 2003101476    A1   20030529    US 2001-21723    20011212
```

=> s ((robertson, d?)) or (robertson d?))/au

UNMATCHED RIGHT PARENTHESIS 'D?))/AU'

The number of right parentheses in a query must be equal to the number of left parentheses.

=> s ((robertson, d?) or (robertson d?))/au

L16 4876 ((ROBERTSON, D?) OR (ROBERTSON D?))/AU

=> s l16 and phytase

L17 2 L16 AND PHYTASE

=> dup rem l17

PROCESSING COMPLETED FOR L17

L18 2 DUP REM L17 (0 DUPLICATES REMOVED)

=> d 1-2 ti

L18 ANSWER 1 OF 2 CAPLUS COPYRIGHT 2004 ACS on STN

TI Enhancing the thermal tolerance and gastric performance of a microbial **phytase** for use as a phosphate-mobilizing monogastric-feed supplement

L18 ANSWER 2 OF 2 CAPLUS COPYRIGHT 2004 ACS on STN

TI New phytases identified by sequence homology and their use in food processing to lower phytic acid content

=> s ((barton, n?) or (barton n?))/au

L19 472 ((BARTON, N?) OR (BARTON N?))/AU

=> s l19 and phytase

L20 8 L19 AND PHYTASE

=> dup rem l20

PROCESSING COMPLETED FOR L20

L21 7 DUP REM L20 (1 DUPLICATE REMOVED)

=> d 1-7 ti

L21 ANSWER 1 OF 7 CAPLUS COPYRIGHT 2004 ACS on STN

TI Sequences of modified recombinant Escherichia coli phytases and methods of making and using them

L21 ANSWER 2 OF 7 CAPLUS COPYRIGHT 2004 ACS on STN

TI Enhancing the thermal tolerance and gastric performance of a microbial **phytase** for use as a phosphate-mobilizing monogastric-feed supplement

L21 ANSWER 3 OF 7 CAPLUS COPYRIGHT 2004 ACS on STN

TI Production, expression and sequence of modified recombinant Escherichia coli **phytase** with enhanced thermostability and proteolytic resistance for use in foodstuffs

L21 ANSWER 4 OF 7 CAPLUS COPYRIGHT 2004 ACS on STN DUPLICATE 1

TI Safety evaluation of a **phytase**, expressed in Schizosaccharomyces pombe, intended for use in animal feed

L21 ANSWER 5 OF 7 CAPLUS COPYRIGHT 2004 ACS on STN

TI New phytases identified by sequence homology and their use in food processing to lower phytic acid content

L21 ANSWER 6 OF 7 CAPLUS COPYRIGHT 2004 ACS on STN

TI Recombinant bacterial phytases and their use for degradation of food phytates.

L21 ANSWER 7 OF 7 CAPLUS COPYRIGHT 2004 ACS on STN  
TI Recombinant bacterial phytases and uses for improved nutritional value of  
phytate-containing foodstuffs

=> d 4 ab

L21 ANSWER 4 OF 7 CAPLUS COPYRIGHT 2004 ACS on STN DUPLICATE 1  
AB BD006 **phytase** is the product of the Escherichia coli B appA gene  
expressed in Schizosaccharomyces pombe strain ASP595-1. This enzyme  
preparation is intended for use in animal feed applications where it improves  
the bioavailability of phosphate for monogastric animals. BD006  
**phytase** was tested as an unrefined (DV006U) and a refined (DV006R)  
preparation for its effects on genotoxicity and in acute, inhalation and  
subchronic toxicity studies. Dosages ranged from 5000 µg/plate for in  
vitro toxicity studies to a high of 2000X for oral in vivo toxicity  
studies. The highest oral dose tested (2000X) is 2000 times the highest  
expected consumption of product by poultry or swine (X=50 units of  
**phytase** per kg bwt/day). There was no genotoxicity or any in vivo  
toxicity reported which could be directly related to systemic effects of  
the product. Other addnl. safety studies conducted were devoid of any  
relevant toxicity. The historic safety profile of the production organism S.  
pombe, and the results of the toxicity studies presented herein, attest to  
the safety of BD006 **phytase** for use in animal feed applications.

=> d 4 so

L21 ANSWER 4 OF 7 CAPLUS COPYRIGHT 2004 ACS on STN DUPLICATE 1  
SO Regulatory Toxicology and Pharmacology (2003), 37(2), 286-292  
CODEN: RTOPDW; ISSN: 0273-2300

=> d 5 ab

L21 ANSWER 5 OF 7 CAPLUS COPYRIGHT 2004 ACS on STN  
AB Novel phytases that can be used to remove phytic acids from foods during  
food processing are identified and characterized. Genes encoding the  
enzymes have been cloned for use in manufacture of the enzyme for industrial  
use. In particular, the **phytase** of the present invention can be  
used in foodstuffs to improve the feeding value of phytate rich  
ingredients.

=> d 5 so

L21 ANSWER 5 OF 7 CAPLUS COPYRIGHT 2004 ACS on STN  
SO PCT Int. Appl., 208 pp.  
CODEN: PIXXD2

=> d 5 pi

L21 ANSWER 5 OF 7 CAPLUS COPYRIGHT 2004 ACS on STN

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2002048332	A2	20020620	WO 2001-US48774	20011212
WO 2002048332	C2	20030424		
WO 2002048332	A3	20030626		

W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN,  
CO, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM,  
HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS,  
LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO,

RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN,  
YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM  
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AT, BE, CH,  
CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR,  
BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG

AU 2002030953	A5	20020624	AU 2002-30953	20011212
US 2003101476	A1	20030529	US 2001-21723	20011212